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CO-Selenium in Fish Tissue: Prediction Equations for Conversion between Whole Body, Muscle, and Eggs

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LIST OF ACRONYMS AND ABBREVIATIONS

BBH. . . . . Black bullhead
BHS. . . . . Bluehead sucker
BT. . . . . Brown trout
cm. . . . . .Centimeter
CCF. . . . . Channel catfish
CWA . . . . Clean Water Act
R^2. . . . . Coefficient of Determination
DOI. . . . . Department of Interior
DW. . . . . Dry Weight
F. . . . . Female
FMS. . . . . .Flannelmouth sucker
g. . . . . Gram
GS. . . . . .Green sunfish
LMB. . . . . .Largemouth bass
M. . . . . Mole
ug/g. . . . . Microgram per gram
ug/l. . . . . .Microgram per liter
mm. . . . . Millimeter
MURR. . . . . University of Missouri Research
                                                                    Reactor
MP. . . . . Muscle plug
Ln. . . . . .Natural log

LIST OF ACRONYMS AND ABBREVIATIONS (cont'd)

NIST. . . . . .National Institute of Standards &
Technology
NIWQP . . . . National Irrigation Water Quality
Program
OE. . . . . .Ovary and egg
ppb. . . . . Parts-per-billion
PL. . . . . .Pooled
PB. . . . . .Post-breeding
PS. . . . . .Pre-spawning
p. . . . . Probability
RTC. . . . . Roundtail chub
n. . . . . Sample size
Se. . . . . Selenium
SMB. . . . . .Smallmouth bass
Spp. . . . . Species
SRM . . . . .Standard Reference Material
Wt. . . . . .Weight
WS. . . . . White sucker
WB. . . . . Whole body

ACKNOWLEDGEMENTS

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ABSTRACT

The lower Gunnison River and the Colorado River are designated critical habitat for two endangered fish species: the Colorado pikeminnow (*Ptychocheilus lucius*) and the razorback sucker (*Xyrauchen texanus*). Approximately 95 river miles of these river segments are state listed as impaired because selenium concentrations exceed the chronic standard for the protection of aquatic life. There is continual need to monitor fish tissue selenium concentrations to assess remediation effectiveness in conjunction with remediation efforts undertaken by the local selenium task force. Muscle plug biopsies provide a non-lethal sampling method to determine selenium residues in endangered fish. There is a need for accurate and validated conversion factors to predict selenium in whole body fish, as well as ovarian (with eggs) concentrations from measured muscle plug residues for comparison to established toxicity thresholds. Selenium concentrations in ovaries and eggs provide the most reliable endpoint to assess risk of reproductive impairment from selenium toxicity. We have developed tissue selenium prediction equations using two fish species; the green sunfish (*Lepomis cyanellus*) and the white sucker (*Catastomus commersoni*). These prediction equations were tested on other fish species to measure performance.

Whole body selenium concentrations predicted from muscle plug concentrations were usually within +/- 30% of the actual measured values. Ovary/egg selenium concentrations predicted from muscle plug concentrations were usually underestimated; especially for black bullhead (*Ictalurus melas*), channel catfish (*Ictalurus punctatus*), brown trout (*Salmo trutta*), and roundtail chub (*Gila robusta*). Fish varied by species when partitioning different selenium loads into their ovary/egg tissues. Black bullheads, channel catfish, and brown trout partition more selenium into ovaries/eggs compared to other species. Green sunfish and white suckers exhibited seasonal differences in ovary/egg selenium concentrations, with pre-spawning females carrying significantly higher selenium loads than post-breeding females. Selenium concentrations in endangered Colorado River fish were at levels known to impair reproduction in other fish species.

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INTRODUCTION

A U.S. Department of the Interior (DOI) irrigation drain-water study of the Uncompahgre Project area and the Grand Valley in western Colorado revealed high selenium concentrations in water, sediment, and biota samples (Butler et al.,1994,1996). Within the study area and pursuant to the Endangered Species Act (ESA), the lower Gunnison River and the Colorado River (including the 100-year floodplain) are designated critical habitat for two endangered fish species: the Colorado pikeminnow (*Ptychocheilus lucius)* and the razorback sucker (*Xyrauchen texanus).* Concern exists that high selenium concentrations are adversely affecting these endangered Colorado River fish (Hamilton, 1999; Hamilton et al., 2001a & b, 2002a, c, &d)**.** In July 1997, the Colorado Water Quality Control Commission (Commission) adopted a 5 ppb aquatic life protection standard for selenium in the Gunnison River Basin (CDPHE, 2001a), and on January 21, 2002, the Commission adopted the same standard for the Colorado River Basin (CDPHE, 2001b). Several stream segments, including the main stem Gunnison River between Delta and the Colorado River confluence, did not meet this 5 ppb selenium standard, and were listed in 1998 pursuant to the CWA 303(d) as impaired water bodies for the State of Colorado. Seasonal water selenium concentrations in some of the Gunnison River tributaries exceed 200 ug/l, with whole body fish samples ranging from 20-50 ug/g dry weight selenium (Butler et al.1994, 1996) and Butler and Osmundson, 2000). Also, several Grand Valley tributaries to the Colorado River and sometimes the main stem Colorado River at the Colorado-Utah State line exceed the 5 ppb standard. These segments were listed as impaired pursuant to the CWA 303(d) in 2002 due to selenium exceedences (www.cdphe.state.co.us). Seasonal water selenium concentrations in Grand Valley drainages which empty into the Colorado River can exceed 100 ug/l, with comparable whole body fish selenium concentrations sometimes exceeding 30 ug/g dry weight (Butler et al.1994, 1996; and Butler and Osmundson, 2000). Thus, a significant portion of critical habitat for the endangered fish, approximately 95 river miles in western Colorado, can exceed the selenium standard for the protection of aquatic life.

Triggered by events at Kesterson National Wildlife Refuge in central California, DOI began the National Irrigation Water Quality Program (NIWQP) in 1985 to investigate whether irrigation-related problems existed at other DOI constructed or managed irrigation projects, which in turn affect national wildlife refuges or other wetland areas for which DOI has responsibility. About 600 irrigation projects and major wildlife resource areas have been constructed or are managed in 17 Western States by DOI bureaus. Of 600 project areas evaluated, five areas indicated potentially serious irrigation and drainage-related water quality problems requiring remediation (Engberg et al. 1998). These five areas include the Gunnison/ Grand Valley Project areas in western Colorado and the Middle Green River Project area in Utah (including Ouray National Wildlife Refuge). Encompassed within the Gunnison/Grand Valley Project area in western Colorado were more than 20 wetland sites identified to need selenium remediation. The Gunnison/Grand Valley core team used a protocol developed by Lemly (1995; 2002), to assess aquatic hazard from selenium at each potential remediation site. Contaminant Specialists in the Grand Junction and Utah field offices also used Lemly's protocol to assess aquatic hazard from selenium for the bottomlands acquisition program component to the Colorado River Recovery Program for the endangered Colorado River fish.

The Lemly (1995; 2002) protocol requires a data set for selenium concentrations measured in five ecosystem components (water, sediment, benthic invertebrates, fish eggs, and bird eggs) for incorporation into the hazard assessment model. Fish eggs are recommended for the model because adverse effects from selenium in fish are associated with lowered reproductive success, that in turn, is associated with high egg selenium concentrations (Lemly, 1996, Maier and Knight, 1994). Recognizing that fish or bird eggs are not always available, Lemly (2002) additionally provided a modified protocol using only four ecosystem components. And Lemly (1995, 2002) provided a conversion factor from fish whole body selenium concentrations to egg concentrations of 3.3 (Whole Body Se X 3.3=Egg Se). Hamilton (per. com., 2002) suggested that the correct conversion factor should actually be 1/0.33 or 3.03. Lemly (1995) estimated this conversion factor from studies where bluegill (*Lepomis macrochirus*) had been exposed to selenite in eastern reservoirs, and has never been validated for other species exposed to

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selenate-laden drain waters in the western U.S. However, preliminary data collected by Grand Junction contaminant specialists indicated this number is probably too high for modeling selenium hazards in the Upper Colorado River Basin. Because mature eggs are a key component in selenium risk assessment, and availability of mature eggs is seasonally limited, there is a need for a reliable conversion factor to estimate selenium residues in eggs from the use of whole body fish or muscle plug residues.

Lemly and Smith (1987) also suggested a conversion factor of 1.667 for estimating selenium concentrations for whole body fish from muscle tissue concentrations (Muscle Se X 1.667= Whole body Se). This conversion factor of 1.667 has also never been validated. Other conversion factors reported in the literature were 2.355 based on data from Adams (1976) for rainbow trout (*Oncorhynchus mykiss)*, and 1.745 based on Lemly (1982) for bluegill and largemouth bass (*Micropterus salmoides*). In contrast, Saiki et al. (1991) found that bluegill and largemouth bass associated with irrigation drain water in central California contained more selenium in muscle tissue than in whole body tissue. Because of the endangered status of the Colorado pikeminnow and razorback sucker, sacrificing these species for tissue residue analysis has been avoided. Waddell and May (1995) developed a non-lethal sampling procedure that uses a minute quantity of muscle tissue (referred to as muscle plugs) to assess selenium residues in the razorback sucker. Osmundson et al. (2000) utilized this technique to determine muscle selenium residues in Colorado pikeminnow.

The U.S. EPA is currently in the process of revising its selenium criteria for the protection of freshwater aquatic life. A peer consultation workshop undertaken by EPA surmised that neither a water nor sediment-based criterion were suitable for determining tissue bioaccumulation of selenium (USEPA, 1998, 2004). Hamilton (2002b) promoted the use of tissue-based criteria and recommended the use of a fish whole body residue of 4 ug/g dry weight as the critical body burden for adverse biological effects, accounting for bioaccumulation of selenium through the diet. Other researchers have recommended that mature ovaries are a more appropriate tissue to use for monitoring selenium residues, because of the link between parental exposure and

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reproductive success (deBruyn et al. 2008, Ohlendorf 2002). Hamilton (2002a) pointed out the fact that ovaries with mature eggs would only be seasonally available for analysis. Hamilton et al. (2001 a & b) also pointed out that some researchers have suggested that little selenium is present in immature gonads during the non-spawning season.

The purpose of this study was threefold: (1) to develop predictive models for estimating selenium concentrations in whole body fish, and to also estimate selenium concentrations in ovary/egg tissues from selenium concentrations in muscle plug biopsies; (2) to investigate how egg selenium concentrations change in pre-spawning fish compared to post-breeding fish, and to determine what relationship exists between egg, whole body, and muscle selenium concentrations before and after spawning; and (3) to be able to assess hazards to native and endangered Colorado River fishes from selenium exposure by using non-lethal muscle plug samples.

Although funding for the NIWQP was eliminated in 2005, Colorado River selenium issues are currently being addressed through the Gunnison and Grand Valley Selenium Task Force, which is a group of private, local, state, and federal interests. To determine if remediation efforts are successful, there is a continued need to monitor fish tissue selenium concentrations within critical habitat in the upper Colorado River basin. Consequently, there also is a need for accurate and validated conversion factors which predict selenium concentrations in whole body fish, as well as in ovaries (with eggs) from measured muscle plug residues.

METHODS

To develop the tissue selenium prediction models, two fish species were used; green sunfish (*Lepomis cyanellus*), which are considered a high selenium accumulator species, and white sucker (*Catostomus commersoni*), which are considered a low selenium accumulator species (Butler et al. 1994; Butler and Osmundson, 2000). Fish were collected using electro-fishing equipment, seining, and nets. The study design goal was to sample both species at two types of

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sites; one site exhibiting high selenium concentrations and one site with relatively low selenium concentrations. Because a range of selenium concentrations was represented in the dataset, fairly robust regression equations could be calculated. Pre-spawning fish were collected when females had ovaries with mature eggs, and post-spawning fish were collected when females had small ovaries with immature eggs. Thus, the experimental design was a 2 X 2 X 2 factorial, with a total of 8 treatments. Study treatments were as follows:

Green Sunfish	White Sucker
High selenium site X Pre-spawning	High selenium site X Pre-spawning
High selenium site X Post-spawning	High selenium site X Post-spawning
Low selenium site X Pre-spawning	Low selenium site X Pre-spawning
Low selenium site X Post-spawning	Low selenium site X Post-spawning

Attempts were made to collect a total of 10 females and 5 males within each treatment for selenium analysis. Both ovaries (containing eggs) were dissected from all females. Muscle plug samples were excised from all fish. After tissue sampling, the rest of the carcass was saved for selenium analysis. Ovaries were removed using chemically cleaned instruments, and placed in chemically clean jars, weighed, and frozen. Muscle plug samples were collected with clean, sterile, disposable 4 or 5-mm biopsy punches according to procedures specified by Williamson (1992), Waddell & May (1995), and Osmundson et al. (2000). Muscle plugs were taken from the dorsal area approximately 1-2 cm either side of the dorsal fin. A different 5-mm sterile biopsy punch was used on a single fish and discarded. Skin was left on the muscle plugs, as it was previously determined there was no difference in selenium concentrations between skin-on verses skin-off muscle plugs (Osmundson, unpublished data). Carcasses were placed in polyethylene bags for storage in a freezer at 20ºC. Muscle plugs were placed in separate sterile cryotubes. All samples were chilled with wet ice in the field, frozen the same day, and kept frozen until preparation for laboratory analysis.

The first regression equation was developed by using whole body (corrected for egg and muscle mass removed) and muscle plug selenium concentrations using combined species and sexes. The second regression equation was developed by using whole body and egg selenium concentrations

using combined species. Initially, equations were developed by lumping data for the two species together to assess whether a reasonably strong "all purpose" equation could be derived. Depending on the coefficient of determination (r^2) generated by combining species, separate species equations may need to be developed; one equation using white suckers representing low accumulator species, and one equation using green sunfish representing high accumulator species. A T-test or ANOVA was used to detect differences in selenium concentrations between males and females for both whole body and muscle concentrations with green sunfish and white suckers analyzed separately. A T-test was used to detect differences between pre- and post-spawning fish in egg, whole body, and muscle plug concentrations for female fish. This was performed separately for green sunfish and white suckers.

To determine the applicability of regression equations developed using green sunfish and white suckers for other species, an attempt was made to collect ten samples of ten different Colorado River fish species and tissue dissected as specified above. These species included native fish found in critical habitat such as, flannelmouth suckers (*Catostomus latipinnis*), bluehead suckers (*Catostomus discobolus*), roundtail chub (*Gila robusta)*, and non-native species such as black bullheads (*Ictalurus melas*), channel catfish (*Ictalurus punctatus*), largemouth and smallmouth bass (*Micropterus salmoides* and *Micropterus dolomieui*), brown trout (*Salmo trutta*), common carp (*Cyprinus carpio*), and fathead minnows (*Pimephales promelas*). Actual selenium concentrations in their tissues were compared to those predicted from the regression equations.

Muscle plugs were taken from wild Colorado pikeminnow and hatchery-stocked razorback suckers to determine accumulation of selenium by fish in critical habitat. Muscle plugs were collected by Colorado River Fisheries Project staff while they were conducting Colorado pikeminnow population surveys. Betadine was used on the endangered Colorado pikeminnow and razorback sucker to disinfect the wound and decrease the chance of infection. Muscle plug concentrations were converted to whole body and egg concentrations using the regression equations developed from this study and Hamilton et al. (2001a & b). These resulting concentrations were then compared to those found in Hamilton (2002 a & b) for association with

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potential adverse effects. Selenium analysis was conducted on the carcass and ovaries by hydride generation atomic absorption spectrometry at the Trace Element Research Laboratory (Texas A&M). Analytical results were reviewed for quality assurance and quality control by the U.S. Fish and Wildlife Services Patuxent Analytical Control Facility (Patuxent, MD). Muscle plug selenium analysis was conducted by neutron activation at the Environmental & Contaminant Research Center (Columbia, MO). Accuracy and precision of the neutron activation method were estimated from Missouri Research Reactor's (MURR's) own internal quality control. MURR conducted accuracy and method precision check by replicate analysis of National Institute of Standards and Technology (NIST) Bovine Liver Standard Reference Material (SRM) 1577. All fish whole body selenium concentrations were calculated by adding ovary/egg selenium back to carcass selenium.

Before statistical analyses, selenium concentration data were ln-transformed to linearize tissue-tissue relationships and stabilize variance for t-test and regression analyses. Statistical analyses included unpaired t-tests to compare selenium concentrations among seasons, sexes, and species. Prediction equations were produced using linear regression analysis.

RESULTS

Quality Assurance/Quality Control

Accuracy and precision for measured spike recovery, reference material, and duplicate sample analysis for selenium analysis by hydride generation at Trace Element Research Laboratory (Texas A&M) on ovary/egg and carcass samples were within acceptable limits. All quality control results for muscle plug analysis using the neutron activation method were considered within acceptable limits as specified by MURR and the Environmental & Contaminant Research Center (Columbia, MO). Recovery of selenium from reference materials analyzed was 100%. Method precision on the MURR replicate analyses was 2.9% relative standard deviation.

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Prediction Equations Using White Suckers and Green Sunfish

Pre-spawning white suckers were collected from late March to April (Appendix 1). Pre-spawning green sunfish were collected from late March through June. Post-breeding white suckers and post-breeding green sunfish were collected during November.

The regression models were developed for the purpose of predicting selenium concentrations in whole body fish, as well as in ovary tissues (with eggs), by using a measured muscle plug selenium concentration. Regression models were built using two species: the green sunfish as a high accumulator species and the white sucker as a low accumulator species. Data from all eight treatments were lumped to determine if a reasonably strong "all purpose" regression could be created for the first model. The first model predicts whole body selenium concentrations by using muscle plug selenium concentrations (Figure 1): The equation was:

$$\text{Ln whole body selenium} = 0.8426 * (\text{Ln muscle plug selenium}) + 0.0231 \quad (1)$$

The coefficient of determination, R^2, of equation 1 was 0.89, and was highly significant ($p<0.0001$). There was no significant difference regarding muscle plug selenium concentrations between males and females for either white suckers (un-paired t-test, $p=0.429$) or green sunfish (un-paired t-test, $p=0.936$). There was also no significant difference regarding whole body selenium concentrations between males and females for either white suckers (un-paired t-test, $p=0.60$) or green sunfish (un-paired t-test, $p=0.23$). Thus, equation 1 was created using both species, both sexes, and both seasons (all eight treatments) for a total sample size of $n=112$, that provided a higher R^2 than using either species alone.
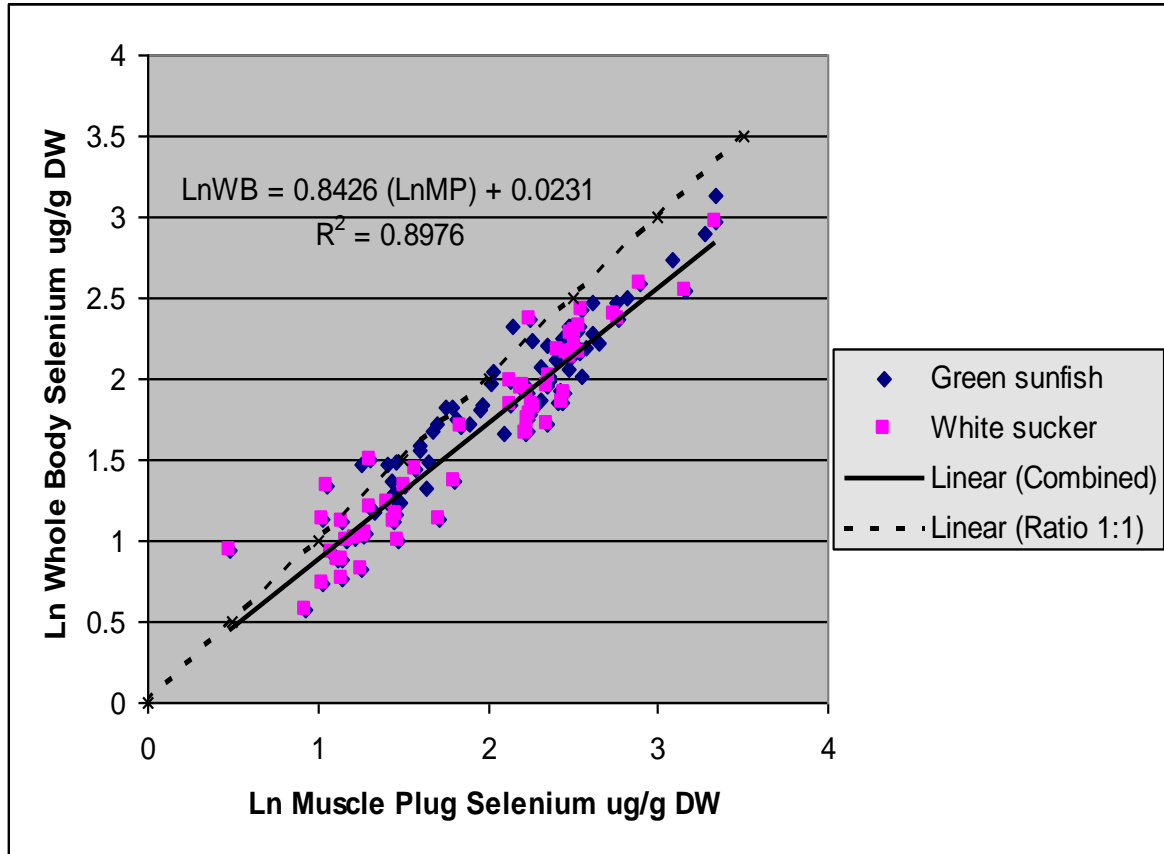
Figure 1. Prediction model for conversion of selenium in muscle plugs to whole body.

The second model, which predicts egg selenium concentrations from muscle plug selenium concentrations, was developed by combining data from green sunfish and white suckers, and combining pre-spawning and post-breeding fish data, for a larger sample size of n=78. The final equation is:

$$\text{Ln egg selenium} = 0.7091 * (\text{Ln muscle plug selenium}) + 0.6733 \qquad (2)$$

with a highly significant (p<0.0001) R^2 of 0.73 (Figure 2). The slope for the green sunfish equation was steeper than for white suckers, which indicates a difference between species regarding selenium deposition in ovaries/eggs compared to muscle. The third equation, which

predicts ovary/egg selenium from whole body selenium, was developed by combining data from both green sunfish and white suckers, and both pre-spawning and post-breeding fish. This equation is:

$$\text{Ln ovary/egg Se} = 0.8123 \text{ (Ln WB selenium)} + 0.6447 \qquad (3)$$

The sample size for equation 3 was n=78, the R^2=0.78. Including the seasonal component of pre-spawning (0=pre-spawning) and post-breeding (1=post-breeding) fish increased the R^2 from 0.78 to 0.81 (equation 4).

$$\text{Ln ovary/egg Se} = 0.77(\text{Ln WB selenium}) - 0.1787 \text{ (0 or 1)} \qquad (4)$$

Including the species component of green sunfish (0=green sunfish) and white suckers (1= white suckers) along with the seasonal component increased the R^2 from 0.81 to 0.83 (equation 5).

$$\text{Ln egg selenium} = 0.7707*(\text{Ln WB selenium}) - 0.1984(0 \text{ or } 1) - 0.1525(0 \text{ or } 1) \qquad (5)$$

Combining species and seasons data in equation 5 provided an improved R^2 over using four separate regression equations that were developed by separating both seasons and species (Figure 3). Regulators monitoring fish tissue to determine if state water quality standards are being met will not always have the luxury of having species specific tissue prediction equations, nor will they be able to monitor at specific times of year to account for the effects of fish reproductive cycles. That is why these several equations are presented in this report.
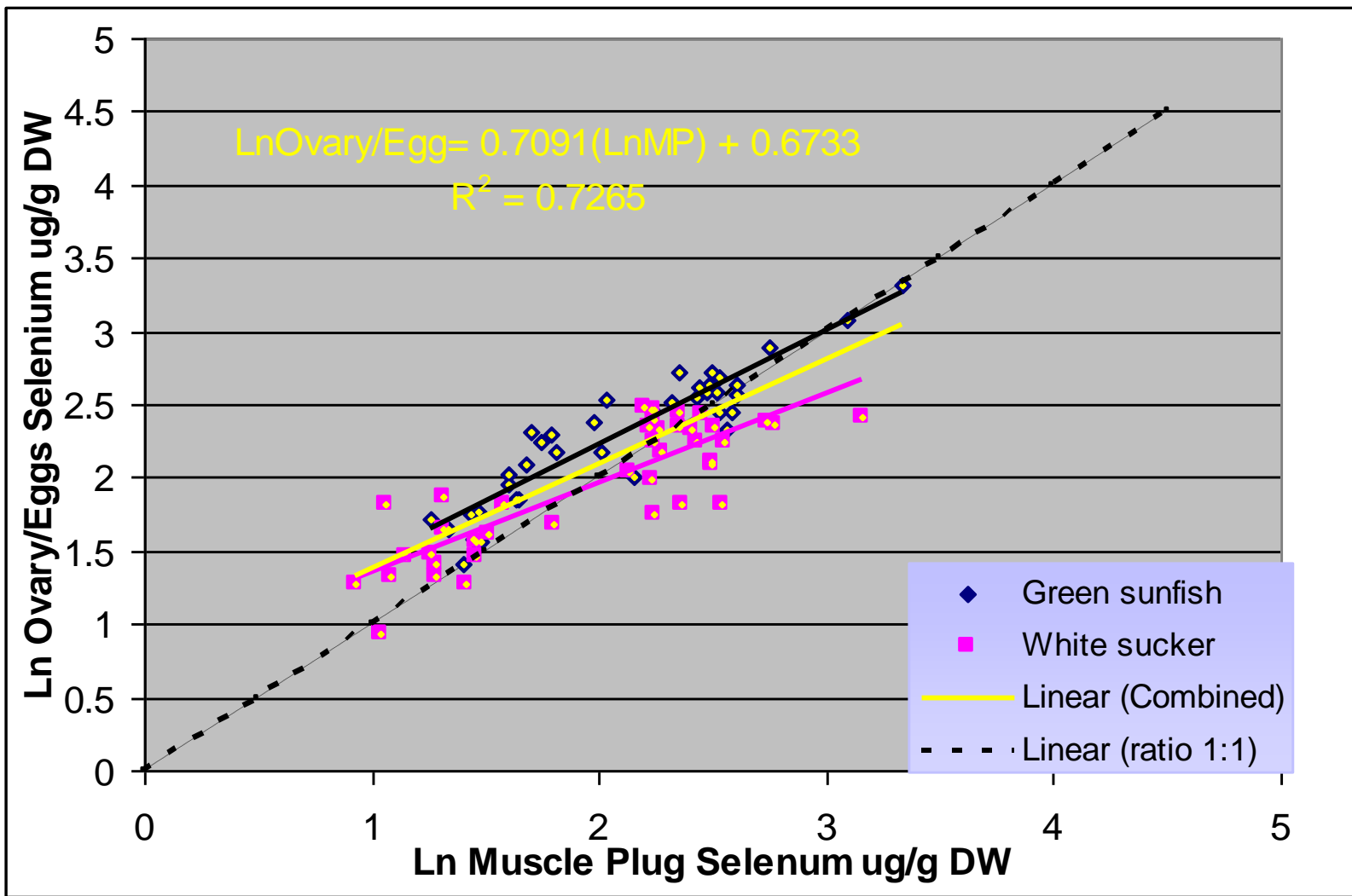
Figure 2. Prediction model for conversion of selenium in muscle plugs to ovaries/eggs
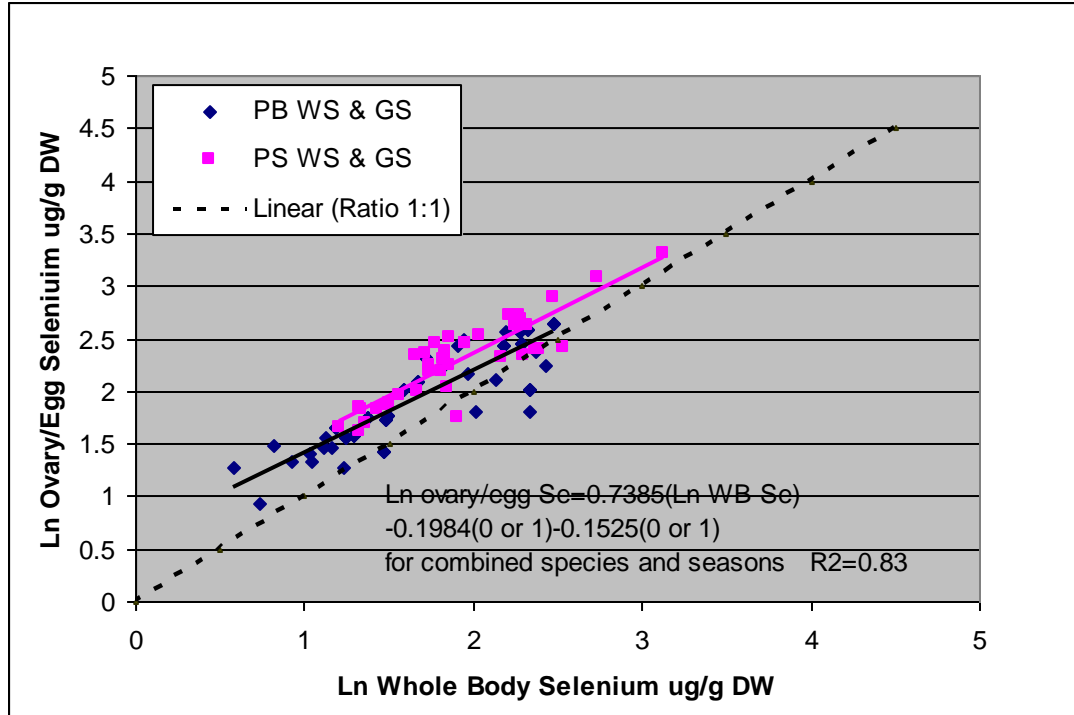
Figure 3. Prediction model for conversion of selenium in whole body fish to ovary/egg tissues.

Tests of Prediction Equations Using Other Species

Selenium concentrations in whole body, muscle plug, and egg samples of other native and non-native fish species found in the Colorado River are displayed in Appendix 2. Selenium whole body concentrations predicted from muscle plug concentrations usually came within +/-30% of the actual measured values (Table 1). Table 2 demonstrates the performance of means for the individual fish species shown in Table 1. Selenium ovary/egg concentrations predicted from muscle plug concentrations were usually underestimated—egregiously so for black bullheads, channel catfish, brown trout, and roundtail chubs (Table 2).

Table 1. Comparison of selenium concentrations predicted from muscle plug selenium concentrations in fish whole body and ovary/eggs to actual values.

Season of Collection[1]	Species[2]	Predicted[3] WB Se	Actual WB Se	Level of Accuracy +/- %	Predicted[3] Egg Se	Actual Egg Se	Level of Accuracy +/-%
PS/March	FMS	3.55	3.1	14.5	4.37	5.9	25.9
PS/March	FMS	3.32	2.63	26.2	3.89	4.13	5.8
PS/April	FMS	5.8	4.48	29.5	5.68	6.19	8.2
PS/April	FMS	4.35	3.5	24.2	4.77	5.73	16.8
PS/April	FMS	2.93	2.95	0.6	4.22	4.02	5
PS/April	FMS	5.02	4.42	13.6	5.62	6.23	9.8
PS/April	FMS	3.93	3.12	26	4.39	4.3	2.1
PB/August	FMS	3.64	2.19	66.2	3.42	3.53	3.1
PB/August	FMS	3.67	1.95	88.2	3.15	4.25	25.9
PB/August	FMS	3.16	2.76	14.5	4.03	6.58	38.8
PB/August	FMS	4.69	4.22	11.1	5.44	4.53	20.1
PB/August	FMS	4.62	4.55	1.5	5.74	5.5	4.4
PS/April	BHS	2.16	1.97	9.6	3.17	4.2	24.5
PS/April	BHS	1.49	1.3	14.6	2.36	2.35	0.4
PS/April	BHS	2.72	2.42	12.4	3.67	4.07	9.8
PS/March	BHS	6.63	5.62	18	6.67	8.05	17.1
PS/March	BHS	4.32	3.91	10.5	5.16	7.1	27.3
PS/April	BHS	2.35	2.11	11.4	3.33	3.69	9.8
PS/April	BHS	2.51	2.18	15.1	3.41	3.99	14.5
PB/August	BHS	2.54	2.11	20.4	3.33	3.92	15.1
PB/August	BHS	3.02	2.43	24.3	3.68	3.48	5.8
PB/August	BHS	3.21	2.13	50.7	3.35	2.7	24.1
PS/March	CCP	13.6	11.7	16.2	11.22	16.3	31.2
PS/March	CCP	6.42	4.78	34.3	5.95	9.37	36.5
PS/March	CCP	5.29	4.1	29	5.33	9.89	46.1
PS/March	CCP	6.13	6.29	2.6	7.22	12.1	40.3
S/May	CCP	16	23.12	30.8	18.17	27.3	33.4
PB/August	CCP	4.97	6.32	21.4	7.25	11.5	37
PB/August	CCP	4.24	4.01	5.7	5.25	9.66	45.7
S/July	CCP	7.68	5.91	30	6.91	11.7	40.9
S/July	CCP	8.51	9.02	5.7	9.33	12.4	24.8
PS/May	BT	3.49	5.02	30.5	6.16	35.6	82.7
PS/June	BT	2.86	4.55	37.1	5.74	32.2	82.2
PS/May	BT	5.09	5.52	7.8	6.58	32.5	79.8
PS/May	BT	3.21	4.3	25.3	5.52	37.8	85.4

Table 1. Comparison of selenium concentrations predicted from muscle plug selenium concentrations in fish whole body and ovary/eggs to actual values.

Season of Collection[1]	Species[2]	Predicted[3] WB Se	Actual WB Se	Level of Accuracy +/- %	Predicted[3] Egg Se	Actual Egg Se	Level of Accuracy +/-%
PS/May	RTC	5.06	6.44	21.4	7.34	15.2	51.7
PS/May	RTC	5.5	6.83	19.5	7.66	14.1	45.7
PS/May	RTC	7.46	8.4	11.2	8.87	17.8	50.2
PS/May	RTC	3.73	4.08	8.6	5.31	7.92	33
PS/May	RTC	4.2	5.27	20.3	6.37	10.8	41.0
PS/May	RTC	5.78	6.55	11.8	7.43	18	58.7
PS/May	RTC	5.56	5.51	0.9	6.58	10.6	37.9
PB/August	RTC	5.87	7.23	18.8	7.97	16.9	52.8
PB/August	RTC	4.01	3.84	4.4	5.09	3.67	38.7
PB/August	RTC	4.6	3.63	26.7	4.89	6.27	22.
PB/July	BBH	5.91	7.31	19.2	8.04	37.3	78.4
PB/July	BBH	3.44	4.83	28.8	5.99	35.4	83.1
PB/July	BBH	3.65	5.47	33.3	6.54	52.8	87.6
PB/July	BBH	5.87	7.64	23.2	8.29	38.7	78.6
PB/August	BBH	6.11	8.59	28.9	9.01	26.4	65.9
PB/August	BBH	4.7	9.61	51.1	9.76	42.8	77.2
PS/June	BBH	6.1	6.61	7.7	7.48	34.3	78.2
PS/June	BBH	7.06	2.03	248	3.24	56.7	94.3
PS/March	BBH	3.97	4.92	19.3	6.07	56	89.2
PS/March	BBH	3	5.3	43.4	6.4	64.3	90.1
S/May	BBH	1.97	2.9	32.1	4.17	28.6	85.4
S/June	BBH	3.94	3.94	0	5.18	12.6	58.9
S/June	BBH	3.41	4.57	25.3	5.76	51.2	88.8
PS/March	BBH	3.74	4.99	25.1	6.13	54.2	88.7
PB/July	CCF	4.42	3.97	11.3	5.21	30.3	82.8
PS/March	CCF	3.17	3.34	5.1	4.61	21.1	78.2
PB/August	CCF	3.04	3.41	10.9	4.68	29.5	84.1
S/June	CCF	3.23	2.63	22.8	3.89	13.7	71.6
PB/August	CCF	3.44	2.04	68.6	3.25	5.82	44.2
PB/August	CCF	1.89	1.88	0.5	3.07	15.9	80.7
PS/May	CCF	3.26	3.35	2.7	4.62	5.01	7.8
PB/August	CCF	1.51	2.35	35.7	3.59	15.2	76.4
S/May	LMB	6.55	7.03	6.8	7.43	11.3	34.3
S/May	SMB	5.53	5.42	2	6.59	6.54	0.77
S/May	SMB	3.22	4.19	23.2	4.49	5.96	24.7
S/May	SMB	4.53	5.07	10.7	5.72	7.13	19.8

Table 1. Comparison of selenium concentrations predicted from muscle plug selenium concentrations in fish whole body and ovary/eggs to actual values.

Season of Collection[1]	Species[2]	Predicted[3] WB Se	Actual WB Se	Level of Accuracy +/- %	Predicted[3] Egg Se	Actual Egg Se	Level of Accuracy +/-%
S/May	SMB	6.05	4.9	23.5	7.03	8.81	20.2
S/May	SMB	5.21	5.51	5.4	6.32	8	21
S/May	SMB	8.19	7.82	4.7	8.71	11	20.8

[1] S=Spawning, PS=Pre-spawning, PB=Post-breeding
[2] FMS=Flannelmouth sucker, BHS=Bluehead sucker, CCP=Common carp, BT=Brown trout, RTC=Roundtail chub, BBH=Black bullhead, CCF=Channel catfish, LMB=Largemouth bass, SMB=Smallmouth bass
[3] Predicted concentrations derived from using regression equations and muscle plug concentrations

Table 2. The performance of means (predicted versus true) selenium concentrations in whole body and ovary/eggs predicted from muscle plug selenium concentrations.

Species[1]	Sample size	Predicted** WB Se	Actual WB Se	Level of Accuracy +/- %	Predicted** Egg Se	Actual Egg Se	Level of Accuracy +/- %
FMS	12	4.06	3.32	22.3	4.56	5.07	10.1
BHS	10	3.1	2.62	18.3	3.81	4.36	12.6
CCP	9	8.09	8.36	3.2	8.51	13.36	36.3
BT	4	3.66	4.85	24.5	6	34.5	82.6
RTC	10	5.18	5.78	10.4	6.75	12.13	44.4
BBH	14	4.49	5.62	20.1	6.58	42.23	84.4
CCF	8	3	2.87	4.5	4.12	17.07	75.9
SMB	6	5.46	5.49	0.55	6.48	7.91	18.1

[1]FMS=Flannelmouth sucker, BHS=Bluehead sucker, CCP=Common carp, BT=Brown trout, RTC=Roundtail chub, BBH=Black bullhead, CCF=Channel catfish, LMB=Largemouth bass, SMB=Smallmouth bass

Fish species differentially partition selenium loads into their ovary/egg tissues relative to muscle or whole body tissues. Black bullheads, channel catfish, and brown trout apparently partition more selenium into ovaries and eggs relative to loads in whole body (Figure 4) or muscle tissue (Figure 5), compared to other species. The ratio of egg to whole-body selenium for black

bullhead showed considerable variability, ranging from 3.1 to 27.9 (Appendix 2). After evaluating 8 different fish species, deBruyn et al. (2008) found rainbow trout (*Oncorhynchus mykiss*) had the highest egg to muscle ratios, and brook trout (*Savelinus fontinalis*) had the
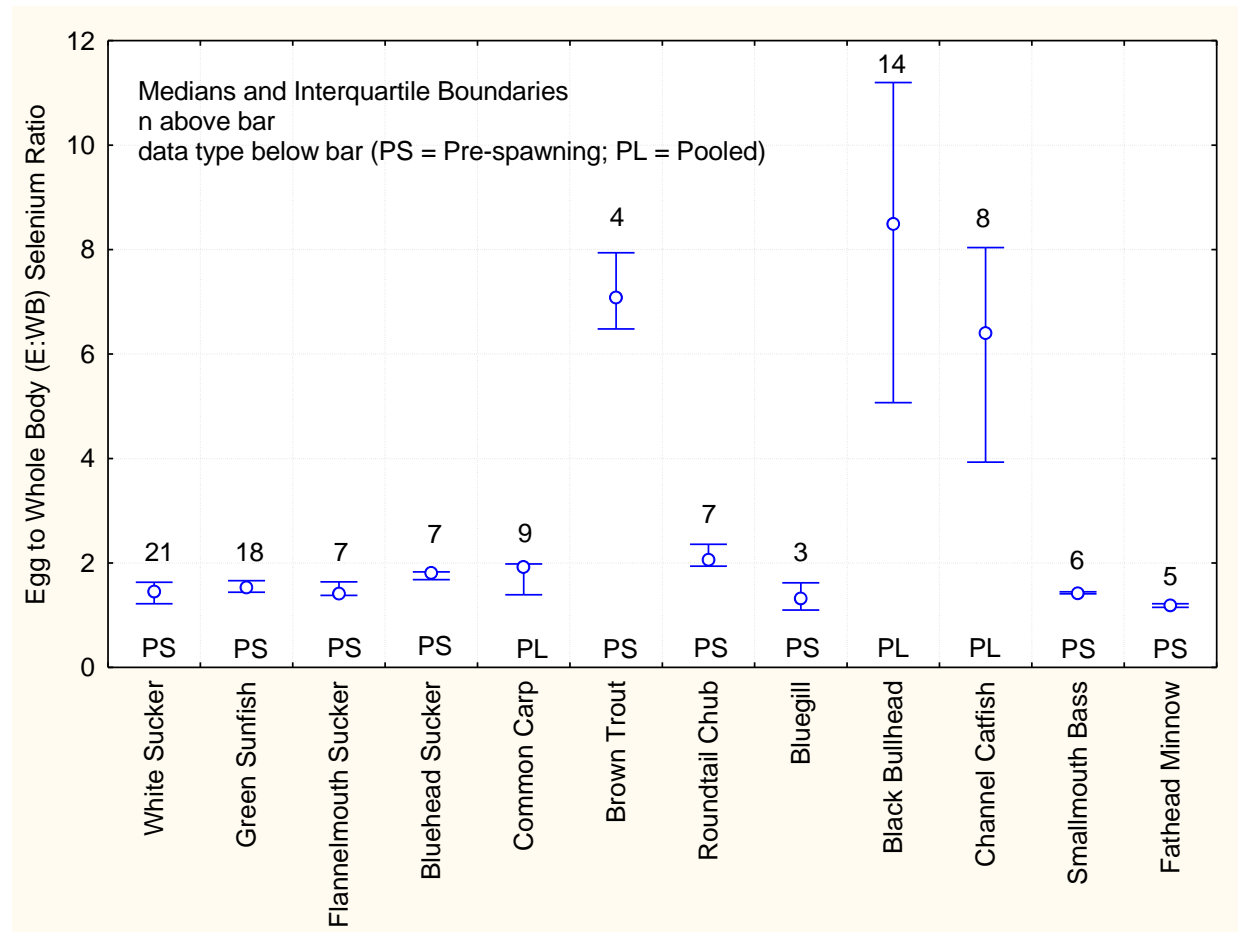
Figure 4. Species variability of selenium partitioning into eggs relative to whole body concentrations.
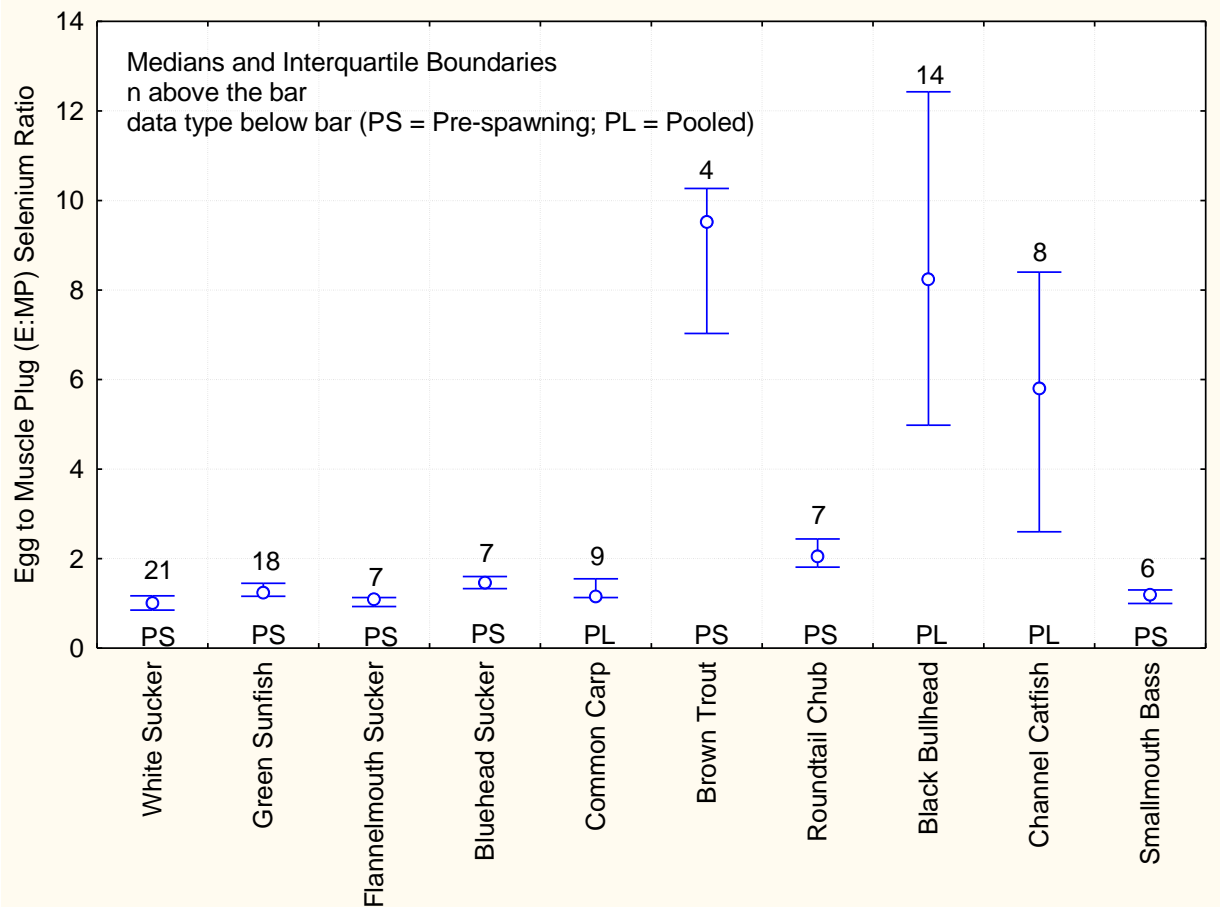
Figure 5. Species variability of selenium partitioning into eggs relative to muscle plug concentrations.

lowest. Doroshov et al. (1992) discussed the cycle of vitellogenesis in catfish. Catfish initiate vitellogenesis in early fall, with vitellogenin synthesis becoming intense in early spring, and spawning occurring in late spring to early summer. Armstrong and Child (1962) noted that channel catfish produce relatively large (3-4 mm) and yolky eggs. Also, their embryos complete major organogenesis during a relatively long period of embryonic development before hatching. Estay (2003) noted that for many salmonid fish species, vitellogenesis can occur over several months prior to spawning, with a relatively large amount of energy-rich yolk deposited into eggs. It is possible that fish with large and yolky eggs deposit more selenium in the eggs than fish with smaller, less yolky eggs. Individual prediction equations were developed for the different species included in this study because of these differences between species (Table 3).

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Table 3. Species specific prediction equations for fish found in the upper Colorado River.

Ln [Y-Value]	Ln [X-Value]	Y-intercept	Slope	r^2	p-value	n	Species[1]	Season[2]
Egg/ovary	Muscle plug	0.6476	0.6589	0.69	0.003	10	BHS	All
		0.6896	0.6955	0.93	0.0004	7		PS
		1.6114	0.6831	0.59	0.043	7	FMS	PS
		0.7344	0.6122	0.72	<0.0001	40	WS	All
		1.1625	0.452	0.6	<0.0001	21		PS
		1.2731	0.5558	0.78	0.0017	9	CCP	All
		0.9775	0.6733	0.84	0.028	5		PS
		-0.1945	1.3966	0.38	0.057	10	RTC	All
		0.815	0.9384	0.67	0.024	7		PS
		0.6683	0.7768	0.86	<0.0001	38	GS	All
		0.7958	0.7476	0.88	<0.0001	18		PS
		1.4793	0.0826	0.84	0.010	6	SM Bass	PS
Egg/ovary	Whole Body	0.6341	0.8713	0.85	0.0002	10	BHS	All
		1.0938	0.4381	0.35	0.044	12	FMS	All
		0.6550	0.8013	0.66	0.027	7		PS
		0.7977	0.6841	0.70	<0.0001	40	WS	All
		1.405	0.5767	0.94	<0.0001	9	CCP	All
		-0.4584	1.6586	0.84	0.0002	10	RTC	All
		0.5297	0.9043	0.85	<0.0001	38	GS	All
		0.5681	0.8785	0.68	0.044	6	SM Bass	PS

Ln [Y-Value]	X-Value	Y-intercept	Slope	r^2	p-value	n	Species[1]	Season[2]
Egg/ovary	Egg Weight	2.3034	0.0032	0.7	0.005	9	CCP	All
		3.9729	-0.1263	0.74	<0.0001	14	BBH	All
		3.1486	-0.0304	0.86	0.001	8	CCF	All

Species[1] BHS=Bluehead sucker, FMS=Flannelmouth sucker, WS=White sucker, CCP=Common carp, RTC=Roundtail chub, GS=Green sucker, Bass=Largemouth and smallmouth combined, BBH=Black bullhead, CCF=Channel catfish
Season[2] PS=Pre-spawning, All=Pre-spawning and post-breeding; Only significant ($p \leq 0.05$) regressions displayed in table.

Seasonal Effects on Selenium Concentrations in Whole Body, Muscle Plug, and Ovary (w/eggs) in White Suckers and Green Sunfish

Whole body samples

There were no significant seasonal differences in whole body selenium concentrations for green sunfish and white sucker males ($p \leq 0.23$, $p \leq 0.95$), or green sunfish and white sucker females ($p \leq 0.09$, $p \leq 0.14$) (Figure 6). However, if all the data is evaluated simultaneously from a meta analysis perspective, the seasonal pattern of declining selenium concentrations in post-breeding samples is consistent across all four comparisons (Figure 6); the probability of that outcome by chance is only $(0.5)(0.5)(0.5)(0.5)=0.0625$. Our data suggest a biologically significant pattern, even though sample sizes were small so statistical power was relatively low. No significant differences were detected in whole body selenium between male and female green sunfish ($p \leq 0.23$), or between male and female white suckers ($p \leq 0.64$) (Figure 6).

Muscle plug samples

Female green sunfish had greater selenium concentrations in muscle plugs taken during the pre-spawning season ($p \leq 0.057$) (Figure 7). Also, during the post-breeding season, male green sunfish had significantly higher selenium in muscle plugs compared to females ($p=0.004$) (Figure 7). In conjunction with the earlier discussion of vitellogenesis, these results indicate that female green sunfish transfer selenium from muscle tissue to ovary/egg tissue during this time. Seasonal and sexual differences among green sunfish samples play a role in whether selenium concentrations fell above or below toxicity guidelines (Lemly 1996, 2002). As with whole body samples, a meta-analysis of the results presented in Figure 7 suggest that selenium accumulation in association with seasonal differences is a biologically real phenomenon.
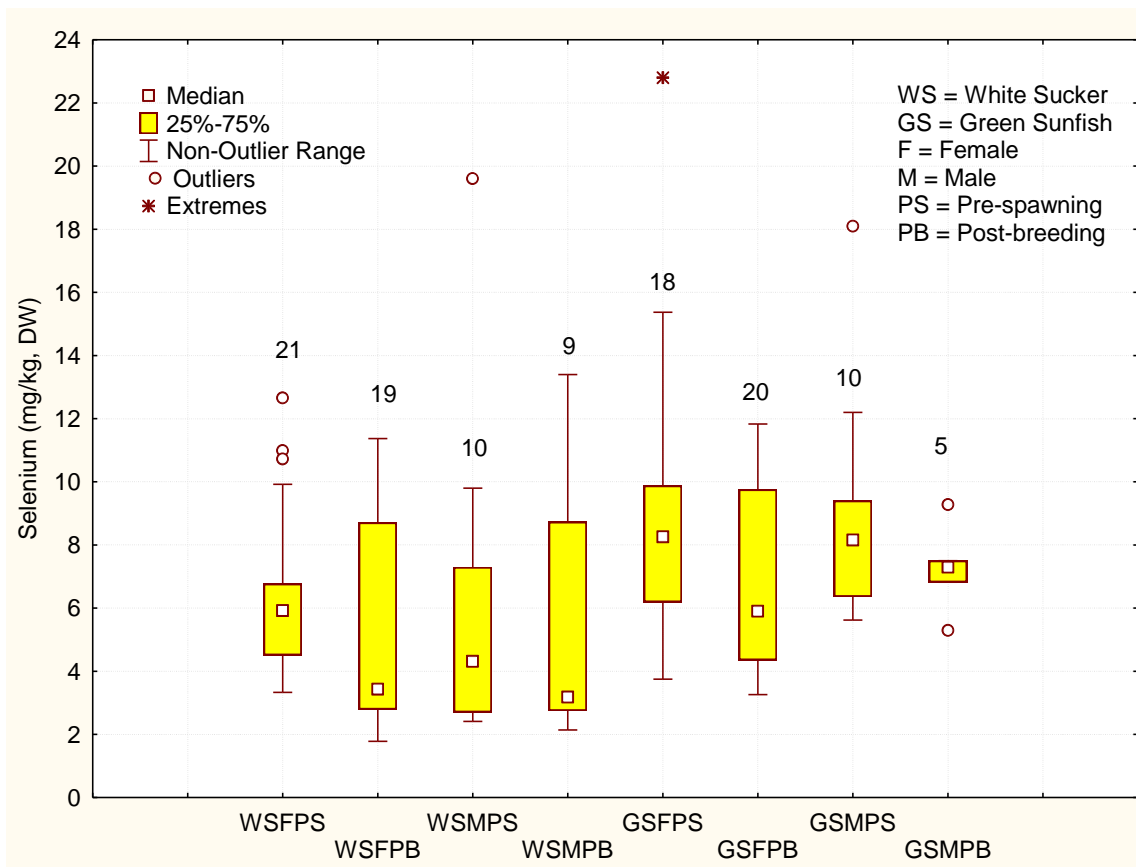
Figure 6. Selenium concentrations in whole body fish samples (species/gender/season).
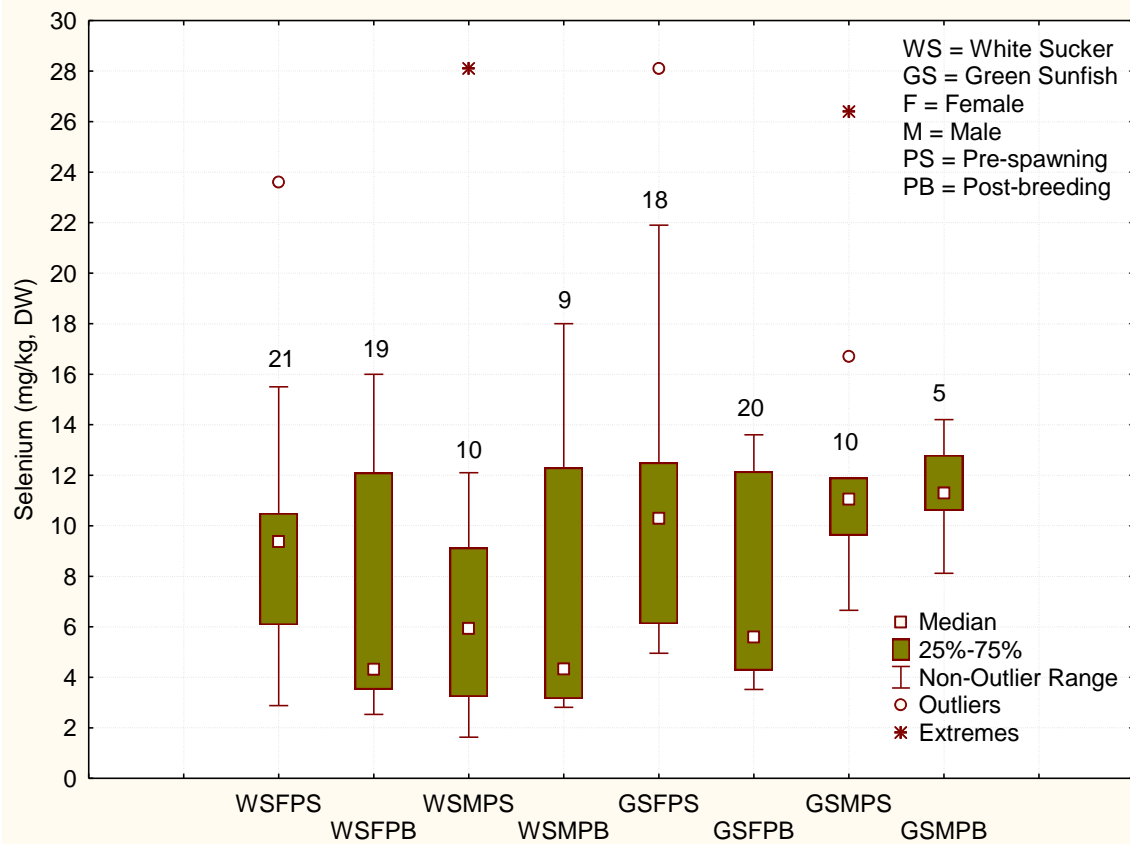
Figure 7. Selenium concentrations in fish muscle plug samples (species/gender/season).

Ovary/egg samples

Our data results show a significant difference in biomass of ovary/egg samples between pre-spawning (μ=59.3 mg) and post-breeding (μ=4.2 mg) white suckers (unpaired t-test, p=0.0003), and between pre-spawning (μ=29.8 mg) and post-breeding (μ=0.35 mg) green sunfish (unpaired t-test, p<0.0001). Seasonal differences in egg selenium concentrations occurred for both green sunfish and white suckers, with pre-spawning females carrying significantly greater selenium loads in ovaries/eggs than post-breeding females (p$\leq$0.01) (Figure 8). Selenium consumed in the diet of female fish is deposited in the eggs, where it is metabolized by larval fish. Kroll and Doroshov (1991), Hamilton et al. (2005) and Janz et al. (2010) included a discussion on egg

development (oogenesis) in fish, and noted that vitellogenesis is the phase of egg development when selenoproteins are incorporated into fish eggs. Vitellogenin is synthesized in the liver, exported into and transported by the blood, and incorporated into the ovarian follicle (Arukwe and Gøksǿyr 2003, Kime 1998). For many fish species, vitellogenesis can occur over several months prior to spawning (Tyler and Sumpter 1996). For these species, dietary intake
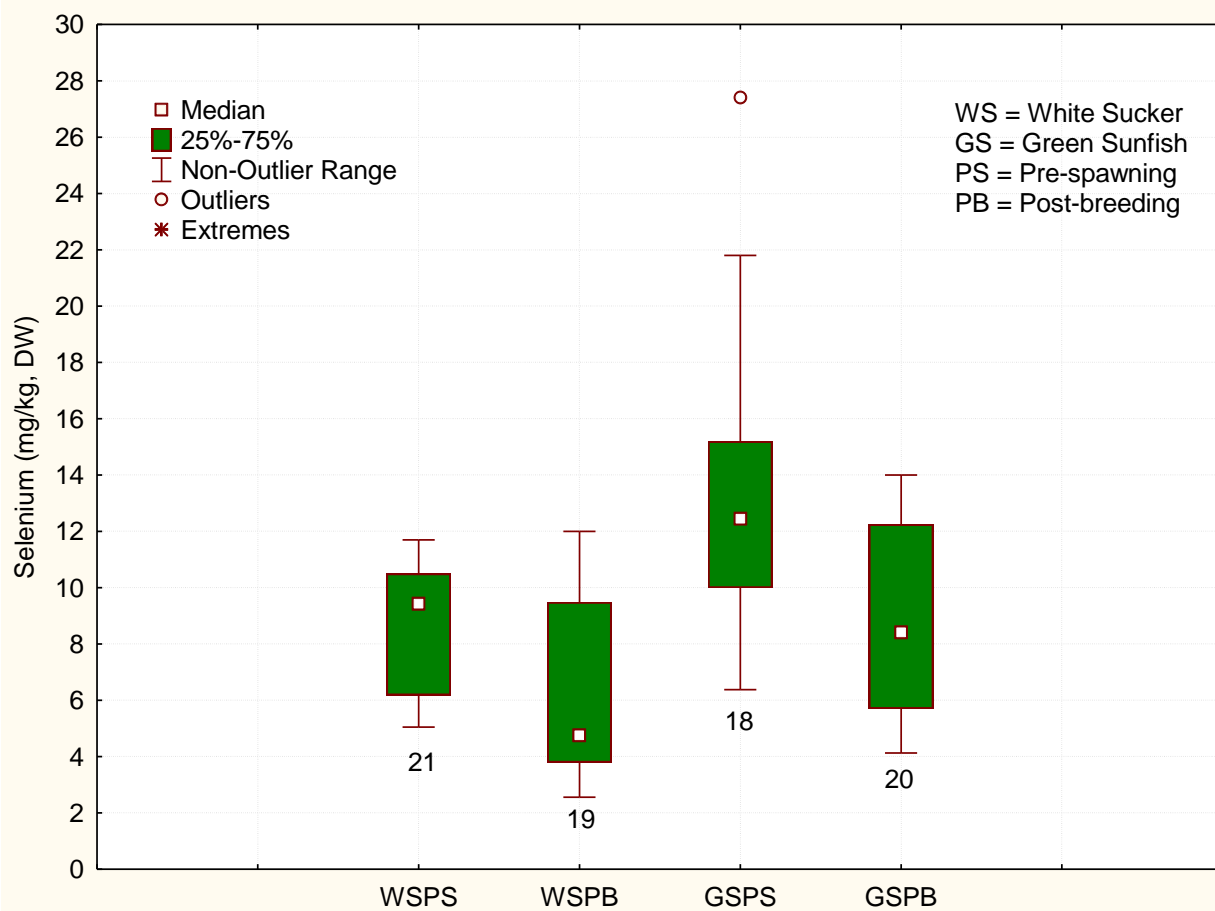
Figure 8. Comparison in ovary/egg selenium concentrations between ripe (pre-spawning) and spent (post-breeding) female fish.

immediately prior to spawning may not have as much of an impact on egg selenium concentrations as does selenium previously stored and mobilized from organs such as liver and muscle. Thus, as noted by Hamilton (2005), high selenium concentrations in food items

consumed in the fall could lead to high selenium concentrations in eggs spawned several months later. Hamilton (2005), and Ng and Idler (1983), discussed the three-part reproductive cycle for rainbow trout (*Oncorhynchus mykiss*): pre-vitellogenesis occurs during December-March, endogenous vitellogenesis occurs during May-July, and exogenous vitellogenesis occurs during July-December. Rainbow trout spawn in May: nine months after the initiation of oogenesis. White suckers spawn once annually in late May, and most likely follow a similar schedule of oogenesis. They also likely follow a similar pattern of selenium deposition into the eggs as do rainbow trout.

Fish species that spawn multiple times in one season have variable cycles of oogenesis, with egg maturation occurring well before, immediately prior to, or during the spawning season (Rinchard and Kestemont 2005, Janz et al. 2010). With this reproductive strategy, selenium in the immediate diet may correlate more closely with selenium concentrations in the eggs. Green sunfish can spawn multiple times during a breeding season in the summer, and so they likely follow this schedule of oogenesis and pattern of selenium deposition into the eggs.

When white sucker samples were divided between pre-spawning and post-breeding fish, and regression analysis was performed using Ln-transformed selenium concentrations in muscle plugs and ovary/egg tissues, the y-intercept, slope, and R^2 changed (Figure 9). Past a certain threshold exposure concentration (the point on the X-axis where the two Ln-Ln regression lines intersect at about 6.4 ppm muscle plug selenium, original units), the ratio of selenium in eggs to muscle plugs switches from being higher in pre-spawning samples to being higher in post-breeding samples. Thus, white suckers respond differentially to "dose"(varying exposures) with respect to partitioning of selenium between muscle tissue and egg tissue as demonstrated in these seasonal changes. For pre-spawning white suckers, there is a diminishing returns allocation of selenium into eggs versus "dose", while for post-breeding white suckers there is an exponential allocation of selenium into eggs.

The seasonal responses for green sunfish with respect to partitioning of selenium between muscle tissue and egg tissue are opposite to the results for white suckers (Figure 10), with the diminishing returns allocation pattern occurring post-breeding and the exponential allocation pattern occurring pre-spawning. This difference between green sunfish and white suckers most likely reflects the difference in breeding strategies between these two species, and different time schedules for selenium deposition into ovaries and eggs.
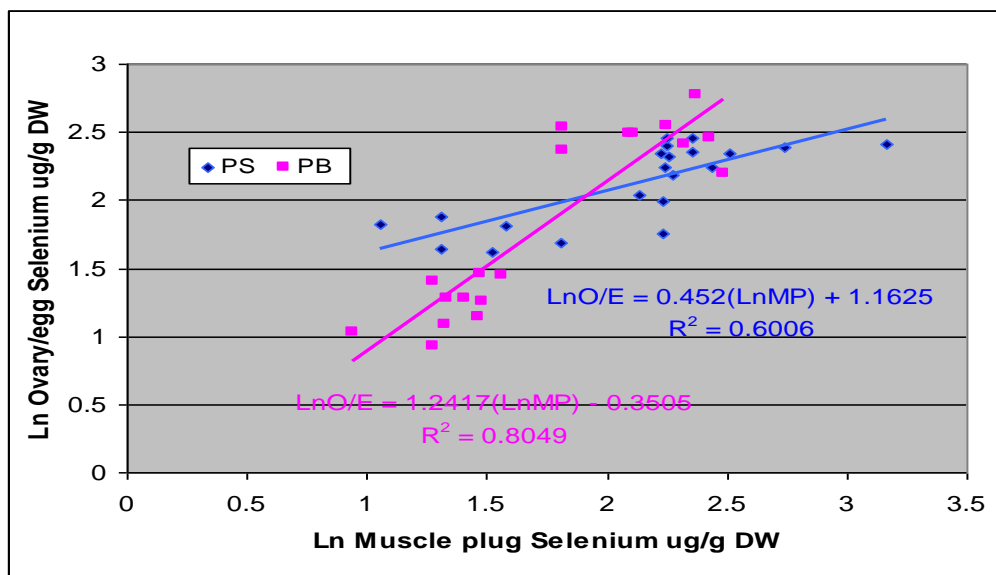
Figure 9. Scatterplot of selenium in ovary/eggs verses muscle plugs in pre-spawning (PS) and post-breeding (PB) white suckers.

Selenium Concentrations in Endangered Colorado River Fish

In 2004 and 2005, muscle plugs were collected from 45 wild Colorado pikeminnow and 49 hatchery-released razorback suckers from the Colorado and Gunnison rivers. Two of 19 Colorado pikeminnow captured in 2004 and 2 of 26 Colorado pikeminnow captured in 2005 had muscle plug selenium concentrations above the 8 ug/g DW Lemly (1996) toxicity guideline for fish muscle (Appendix 3). These four Colorado pikeminnow were all captured from the 18- mile reach of the Colorado River below the Gunnison River confluence, where the state selenium
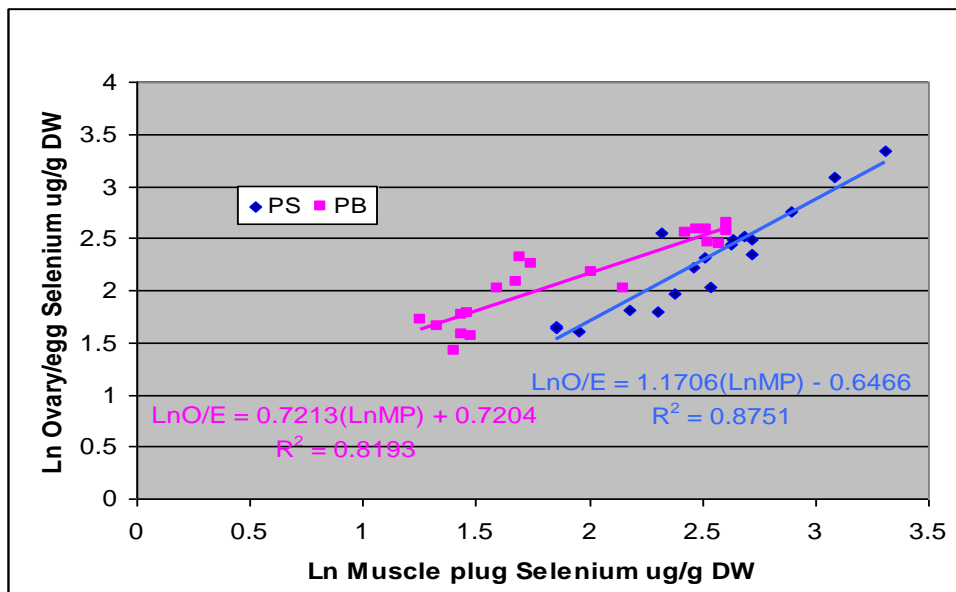
Figure 10. Scatterplot of selenium in ovary/eggs verses muscle plugs in pre-spawning (PS) and post-breeding (PB) green sunfish.

water quality standard of 5 µg/l is exceeded. Egg selenium concentrations were estimated from muscle plug concentrations by using the developed prediction model from white suckers and green sunfish (equation 2), and also by using the PS RTC equation in Table 2. Egg selenium concentrations were also estimated using a prediction equation developed with Colorado pikeminnow data from Buhl and Hamilton (2000) (LnEgg=2.2213(LnMP)+0.1489). Almost all estimated egg concentrations fall in the low hazard category using estimates made with equation 2, but fall in the moderate hazard category using estimates made with the PS RTC equation. The fact that muscle plug concentrations for the four Colorado pikeminnow were above toxicity guidelines, but egg concentrations were predicted to be in the low hazard category indicates that the developed prediction equation using green sunfish and white suckers underestimated egg concentrations for this species, and thus underestimated risk from selenium toxicity. The use of the PS RTC equation may be more accurate for assessing egg selenium concentrations for Colorado pikeminnow. A species specific prediction equation is needed to accurately estimate potential selenium toxicity for the endangered Colorado pikeminnow.

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Razorback suckers previously stocked in the Colorado and Gunnison Rivers were recaptured in 2004 and 2005, after they had been at large for at least 8 months. Of 49 total razorback sucker muscle plugs, 13 had muscle plug selenium concentrations exceeding the 8 ug/g DW Lemly (1996) toxicity guideline for fish muscle tissue (Appendix 4). Egg selenium concentrations were estimated from muscle plug concentrations using a linear regression model (LnEgg selenium=1.3(Ln muscle plug selenium)-0.0575) developed for this species from data presented in Hamilton (2001a & b). This model produced a significant (p<0.0001, R^2=0.79, n=36) correlation. Of 49 razorback suckers captured from the Colorado and Gunnison rivers, estimated egg concentrations for 22 fish fell into the moderate to high risk hazard category (Lemly 1996).

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

Different fish species partition different selenium loads into their ovary/egg tissues. Black bullheads, channel catfish, and brown trout apparently partition more selenium into ovaries/eggs compared to other species. Because of inter-specific variability in tissue-tissue relationships, we recommend calculating species-specific **regression equations used for estimating selenium concentrations in ovary/egg tissue from whole body or muscle plugs.** Also, because there can be tremendous intra-species variability as to how much selenium is stored in ovary/egg tissue (as demonstrated by black bullhead), **we recommend that tissue to tissue extrapolations be site specific**. There were seasonal differences in ovary/egg selenium concentrations for both green sunfish and white suckers, with pre-spawning females carrying significantly higher selenium loads than post-breeding females. To fully assess risk to fish from selenium concentrations in ovary/egg tissues, **we recommend that fish be collected immediately prior to spawning, when ovary/egg biomass and selenium concentrations are highest**. **We recommend that the design of a monitoring program using muscle plug samples incorporate potential seasonal and sexual differences in muscle plug selenium concentrations (as displayed in muscle tissue variations by green sunfish),** to assess risk to fish from selenium exposure, and to monitor effectiveness of selenium remediation efforts. Selenium concentrations found in several

endangered razorback suckers collected in the Colorado and Gunnison rivers are at levels known to impair reproduction in other species of fish. **This observation reinforces the need to remediate high selenium concentrations in the Gunnison and Colorado rivers to attain approved water quality criteria in order to improve endangered fish recruitment and assist in species recovery.**

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Appendix 1. Selenium concentrations in whole body (WB), ovary and egg (OE), and muscle plug (MP) samples taken from green sunfish (GS) and white suckers (WS) during the pre-spawning (PS) and post-breeding (PB) seasons.

Season	Species	Sex	Hi/Lo	Whole body(WB)	Ovary/ egg (OE)	Muscle plug (MP)	Total Weight	Egg Weight	OE:WB ratio	OE:MP ratio
PS	WS	F	HI	3.81	6.18	2.88	1595.3	218.8	1.6	2.14
PS	WS	F	HI	4.21	6.15	4.83	814.9	85.6	1.5	1.27
PS	WS	F	HI	3.33	5.18	3.7	1155.6	165.4	1.6	1.40
PS	WS	F	HI	4.51	6.53	3.7	799.9	102.4	1.4	1.76
PS	WS	F	HI	6.32	7.69	8.42	480.5	38.9	1.2	0.91
PS	WS	F	HI	6.77	5.77	9.36	172.1	4.8	0.9	0.62
PS	WS	F	HI	10.99	10.9	15.5	209.6	13	1.0	0.70
PS	WS	F	HI	12.65	11.2	23.6	179.7	7.3	0.9	0.47
PS	WS	F	HI	5.72	9.43	9.37	167	16.9	1.6	1.01
PS	WS	F	HI	3.92	5.39	6.07	755.9	75	1.4	0.89
PS	WS	F	HI	3.79	5.05	4.56	166	22.1	1.3	1.11
PS	WS	F	LO	9.92	10.4	12.3	461.9	58.5	1.0	0.85
PS	WS	F	LO	5.28	10.4	9.23	678	82.7	2.0	1.13
PS	WS	F	LO	10.72	11	9.44	893.1	37.8	1.0	1.17
PS	WS	F	LO	5.92	11.7	9.44	1114.6	143.4	2.0	1.24
PS	WS	F	LO	7.03	11.6	10.5	781	36.8	1.7	1.10
PS	WS	F	LO	6.4	9.44	11.4	689.7	38.9	1.5	0.83
PS	WS	F	LO	6.27	10.2	9.57	534.1	13.2	1.6	1.07
PS	WS	F	LO	5.33	7.34	9.29	439.4	10.8	1.4	0.79
PS	WS	F	LO	6.15	8.87	9.75	790.7	62.2	1.4	0.91
PS	WS	F	LO	5.59	10.5	10.5	458.2	11.8	1.9	1.00
PB	WS	F	HI	8.8	10.2	11.1	140	17.6	1.2	0.92
PB	WS	F	HI	8.71	8.12	12.1	104.6	9.7	0.9	0.67
PB	WS	F	HI	11.37	9.49	12.8	115.5	1.9	0.8	0.74
PB	WS	F	HI	10.7	10.7	16	146	4.2	1.0	0.67
PB	WS	F	HI	8.44	8.25	12.1	47.1	1.7	1.0	0.68
PB	WS	F	HI	6.95	12	8.99	352.6	16.5	1.7	1.33
PB	WS	F	HI	7.49	6.13	10.6	134.3	2	0.8	0.58
PB	WS	F	HI	10.28	6.13	12.6	775.1	2.9	0.6	0.49
PB	WS	F	HI	6.73	11.3	11.6	329.8	21.6	1.7	0.97
PB	WS	F	LO	2.09	2.56	2.81	311.2	7.7	1.2	0.91
PB	WS	F	LO	1.78	3.57	2.53	569.6	20	2.0	1.41
PB	WS	F	LO	3.21	4.35	4.31	403.3	7.1	1.4	1.01
PB	WS	F	LO	2.27	4.41	3.52	427.5	15	1.9	1.25
PB	WS	F	LO	3.08	4.76	4.27	365.4	11.4	1.5	1.11
PB	WS	F	LO	3.04	4.31	3.14	102.7	8.1	1.4	1.37
PB	WS	F	LO	2.79	4.07	3.58	427.5	14.8	1.5	1.14
PB	WS	F	LO	2.51	3.77	2.95	310.3	16.3	1.5	1.28
PB	WS	F	LO	3.43	3.58	4.09	215.1	8.5	1.0	0.88
PB	WS	F	LO	2.83	3.79	3.59	283.5	5	1.3	1.06

Appendix 1. Selenium concentrations in whole body (WB), ovary and egg (OE), and muscle plug (MP) samples taken from green sunfish (GS) and white suckers (WS) during the pre-spawning (PS) and post-breeding (PB) seasons.

Season	Species	Sex	Hi/Lo	Whole body(WB)	Ovary/ egg (OE)	Muscle plug (MP)	Total Weight	Egg Weight	OE:WB ratio	OE:MP ratio
PS	GS	F	HI	22.8	27.4	28.1	5	0.1	1.2	0.98
PS	GS	F	HI	8.79	10.2	12.9	55.1	0.9	1.2	0.79
PS	GS	F	HI	15.37	21.8	21.9	25	0.3	1.4	1.00
PS	GS	F	HI	4.75	7.03	4.95	13	0.2	1.5	1.42
PS	GS	F	HI	5.74	8.86	6.11	17.4	0.4	1.5	1.45
PS	GS	F	HI	4.43	6.39	5.19	13.4	0.2	1.4	1.23
PS	GS	F	HI	3.75	6.38	5.14	105.8	1.4	1.7	1.24
PS	GS	F	HI	11.9	18.1	15.7	9.5	0.1	1.5	1.15
PS	GS	F	HI	6.43	12.3	10.1	35	0.7	1.9	1.22
PS	GS	F	HI	9.51	13.8	11.5	51.2	0.7	1.5	1.20
PS	GS	F	LO	9.13	15.2	10.5	23.3	3.7	1.7	1.45
PS	GS	F	LO	6.24	10.8	7.2	26.1	1.2	1.7	1.50
PS	GS	F	LO	7.04	11.7	9.26	39.3	2.3	1.7	1.26
PS	GS	F	LO	7.72	12.6	7.65	28.3	2.1	1.6	1.65
PS	GS	F	LO	6.19	9.99	5.99	32.2	4.1	1.6	1.67
PS	GS	F	LO	10.2	13.9	12	23.8	1.3	1.4	1.16
PS	GS	F	LO	9.71	15.2	12.1	19	0.7	1.6	1.26
PS	GS	F	LO	9.88	14.7	12.5	13.7	1.2	1.5	1.18
PB	GS	F	HI	7.18	8.78	7.49	12.1	0.1	1.2	1.17
PB	GS	F	HI	8.99	12.9	11.3	23.3	0.3	1.4	1.14
PB	GS	F	HI	9.7	13.1	13.6	11.5	0.1	1.4	0.96
PB	GS	F	HI	8.89	11.5	13.2	16	0.1	1.3	0.87
PB	GS	F	HI	9.81	13.2	12.4	14.8	0.2	1.3	1.06
PB	GS	F	HI	9.87	11.6	12.5	11.1	0.1	1.2	0.93
PB	GS	F	HI	10.27	7.5	8.59	35.9	0.4	0.7	0.87
PB	GS	F	HI	5.34	8.05	5.34	17.4	0.2	1.5	1.51
PB	GS	F	HI	10.14	13.2	11.9	12.7	0.2	1.3	1.11
PB	GS	F	HI	11.83	14	13.6	9.8	0.1	1.2	1.03
PB	GS	F	LO	3.26	5.22	3.79	36	0.5	1.6	1.38
PB	GS	F	LO	3.95	5.8	4.22	30.2	1	1.5	1.37
PB	GS	F	LO	4.33	4.13	4.08	49	0.8	1.0	1.01
PB	GS	F	LO	3.65	4.86	4.23	40.6	0.4	1.3	1.15
PB	GS	F	LO	6.22	9.47	5.74	17.2	0.4	1.5	1.65
PB	GS	F	LO	3.46	4.75	4.41	31.8	0.6	1.4	1.08
PB	GS	F	LO	4.38	5.6	3.52	26.3	0.5	1.3	1.59
PB	GS	F	LO	5.59	10.1	5.45	19.1	0.3	1.8	1.85
PB	GS	F	LO	4.87	7.54	4.95	17	0.2	1.5	1.52
PB	GS	F	LO	4.44	5.9	4.32	17.2	0.4	1.3	1.37
PS	GS	M	HI	7.96		10.1	49.9			
PS	GS	M	HI	7.87		11.9	49.6			

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Appendix 1. Selenium concentrations in whole body (WB), ovary and egg (OE), and muscle plug (MP) samples taken from green sunfish (GS) and white suckers (WS) during the pre-spawning (PS) and post-breeding (PB) seasons.

Season	Species	Sex	Hi/Lo	Whole body(WB)	Ovary/ egg (OE)	Muscle plug (MP)	Total Weight	Egg Weight	OE:WB ratio	OE:MP ratio
PS	GS	M	HI	6.36		11.1	57.7			
PS	GS	M	HI	8.67		11.8	56			
PS	GS	M	HI	8.34		11	48.9			
PS	GS	M	LO	6.08		7.08	65.9			
PS	GS	M	LO	5.62		6.65	36.8			
PS	GS	M	LO	18.1		26.4	32.6			
PS	GS	M	LO	9.4		9.62	25.8			
PS	GS	M	LO	12.2		16.7	59.8			
PB	GS	M	HI	5.29		8.12	44.7			
PB	GS	M	HI	7.3		10.6	27.6			
PB	GS	M	HI	9.28		14.2	61.7			
PB	GS	M	HI	6.82		11.3	67.1			
PB	GS	M	HI	7.5		12.8	73.7			
PS	WS	M	LO	3.1		5.58	722.9			
PS	WS	M	LO	5.51		6.29	292.6			
PS	WS	M	LO	7.02		9.13	406.4			
PS	WS	M	LO	7.3		8.47	546.3			
PS	WS	M	LO	2.41		3.04	542.7			
PS	WS	M	HI	2.72		4.39	954.9			
PS	WS	M	HI	2.7		3.23	990.9			
PS	WS	M	HI	2.55		1.63	896.9			
PS	WS	M	HI	19.6		28.1	73.7			
PS	WS	M	HI	9.8		12.1	111.7			
PB	WS	M	HI	8.69		11.8	118.3			
PB	WS	M	HI	8.73		12.6	103.6			
PB	WS	M	HI	9.08		12.3	63.7			
PB	WS	M	HI	13.4		18	83.9			
PB	WS	M	LO	3.12		2.81	356.6			
PB	WS	M	LO	2.43		3.15	552.1			
PB	WS	M	LO	2.14		3.14	382.2			
PB	WS	M	LO	3.18		4.32	407.7			
PB	WS	M	LO	2.75		3.4	319			

Appendix 2. Selenium concentrations in whole body (WB), ovary and egg (Egg), and muscle plug (MP) samples taken from native and non-native fish species in the Colorado River.

Collection Date	Species[1]	WB Se ug/g DW	Egg Se ug/g DW	MP Se ug/g DW	Egg/WB	MP/WB	Egg/MP	WB Wt.(g)	Egg Wt.(g)
3/25/2004	FMS	3.1	5.9	4.09	1.90	1.32	1.44	1188	49.0
3/25/2004	FMS	2.63	4.13	3.79	1.57	1.44	1.09	1072	54.0
4/15/2004	FMS	4.48	6.19	7.28	1.38	1.63	0.85	1055	52.0
4/16/2004	FMS	3.5	5.73	5.23	1.64	1.49	1.10	1005	43.0
4/16/2004	FMS	2.95	4.02	3.56	1.36	1.21	1.13	1097	65.0
4/16/2004	FMS	4.42	6.23	6.15	1.41	1.39	1.01	1069	60.0
4/16/2004	FMS	3.12	4.3	4.63	1.38	1.48	0.93	795	38.0
8/21/2002	FMS	2.19	3.53	4.23	1.61	1.93	0.83	989	21.0
8/21/2002	FMS	1.95	4.25	4.28	2.18	2.19	0.99	644	1.0
8/21/2002	FMS	2.76	6.58	3.57	2.38	1.29	1.84	1034	13.0
8/21/2002	FMS	4.22	4.53	5.72	1.07	1.36	0.79	624	1.0
8/21/2002	FMS	4.55	5.5	5.6	1.21	1.23	0.98	709	9.0
4/16/2004	BHS	1.97	4.2	2.3	2.13	1.17	1.83	759	29.0
4/16/2004	BHS	1.3	2.35	1.47	1.81	1.13	1.60	518	20.0
4/16/2004	BHS	2.42	4.07	3.07	1.68	1.27	1.33	572	27.0
3/15/2005	BHS	5.62	8.05	8.57	1.43	1.52	0.94	296	18.0
5/19/2005	BHS	3.91	7.1	5.16	1.82	1.32	1.38	468	39.0
4/15/2004	BHS	2.11	3.69	2.52	1.75	1.19	1.46	584	48.0
4/15/2004	BHS	2.18	3.99	2.72	1.83	1.25	1.47	604	69.0
8/21/2002	BHS	2.11	3.92	2.76	1.86	1.31	1.42	511	3.0
8/21/2002	BHS	2.43	3.48	3.04	1.43	1.25	1.14	611	2.0
8/21/2002	BHS	2.13	2.7	3.64	1.27	1.71	0.74	599	4.0
3/15/2005	CCP	11.7	16.3	20	1.39	1.71	0.82	1675	68.0
3/15/2005	CCP	4.78	9.37	8.24	1.96	1.72	1.14	952	39.0
3/15/2005	CCP	4.1	9.89	6.56	2.41	1.60	1.51	691	35.0
3/15/2005	CCP	6.29	12.1	7.81	1.92	1.24	1.55	1659	52.0
5/19/2005	CCP	23.12	27.3	24.2	1.18	1.05	1.13	2149	280.0
8/21/2002	CCP	6.32	11.5	6.1	1.82	0.97	1.89	2173	138.0
8/21/2002	CCP	4.01	9.66	5.06	2.41	1.26	1.91	1405	10.0
7/8/2002	CCP	5.91	11.7	10.2	1.98	1.73	1.15	1306	11.0
7/8/2002	CCP	9.02	12.4	11.5	1.37	1.27	1.08	1819	17.0
8/1/2005	BT	5.02	35.6	4.01	7.09	0.80	8.88	418	12.0
6/28/2005	BT	4.55	32.2	3.17	7.08	0.70	10.16	360	5.0
5/19/2005	BT	5.52	32.5	6.27	5.89	1.14	5.18	235	1.0
7/20/2005	BT	4.3	37.8	3.64	8.79	0.85	10.38	301	5.0
5/26/2005	RTC	6.44	15.2	6.22	2.36	0.97	2.44	507	60.0
5/26/2005	RTC	6.83	14.1	6.87	2.06	1.01	2.05	603	63.0

Appendix 2. Selenium concentrations in whole body (WB), ovary and egg (Egg), and muscle plug (MP) samples taken from native and non-native fish species in the Colorado River.

Collection Date	Species[1]	WB Se ug/g DW	Egg Se ug/g DW	MP Se ug/g DW	Egg/WB	MP/WB	Egg/MP	WB Wt.(g)	Egg Wt.(g)
5/26/2005	RTC	8.4	17.8	9.84	2.12	1.17	1.81	511	43.0
5/31/2005	RTC	4.08	7.92	4.34	1.94	1.06	1.82	485	74.0
5/31/2005	RTC	5.27	10.8	5	2.05	0.95	2.16	422	55.0
5/19/2005	RTC	6.55	18	7.29	2.75	1.11	2.47	517	39.0
5/27/2005	RTC	5.51	10.6	6.96	1.92	1.26	1.52	284	22.0
8/21/2002	RTC	7.23	16.9	7.42	2.34	1.03	2.28	247	3.0
8/21/2002	RTC	3.84	3.67	5.74	0.96	1.49	0.64	311	1.0
8/21/2002	RTC	3.63	6.27	5.56	1.73	1.53	1.13	376	6.0
5/26/2005	BG	8.78	9.67	12.9	1.10	1.47	0.75	38	3.0
5/15/2002	BG	5.02	6.65	---	1.32	---	---	127	2.0
5/15/2002	BG	6.99	11.3	---	1.62	---	---	108	2.0
7/20/2005	BH	7.31	37.3	7.49	5.10	1.02	4.98	108	1.0
7/20/2005	BH	4.83	35.4	3.94	7.33	0.82	8.98	91	0.5
7/20/2005	BH	5.47	52.8	4.26	9.65	0.78	12.39	137	1.0
7/20/2005	BH	7.64	38.7	7.42	5.07	0.97	5.22	139	3.0
8/9/2005	BH	8.59	26.4	7.82	3.07	0.91	3.38	178	6.0
8/9/2005	BH	9.61	42.8	5.7	4.45	0.59	7.51	101	1.0
6/28/2005	BH	6.61	34.3	7.77	5.19	1.18	4.41	114	2.0
6/28/2005	BH	2.03	56.7	9.22	27.93	4.54	6.15	191	1.0
3/14/2005	BH	4.92	56	4.67	11.38	0.95	11.99	149	0.5
3/14/2005	BH	5.3	64.3	3.35	12.13	0.63	19.19	123	0.3
5/15/2006	BH	2.9	28.6	2.04	9.86	0.70	14.02	202	2.0
6/18/2006	BH	3.94	12.6	4.63	3.20	1.18	2.72	224	11.0
6/19/2006	BH	4.57	51.2	3.91	11.20	0.86	13.09	171	3.0
3/24/2003	BH	4.99	54.2	4.36	10.86	0.87	12.43	186	1.0
7/20/2005	CCF	3.97	30.3	5.31	7.63	1.34	5.71	581	2.0
3/15/2005	CCF	3.34	21.1	3.58	6.32	1.07	5.89	573	1.0
8/5/2005	CCF	3.41	29.5	3.41	8.65	1.00	8.65	599	4.0
6/28/2005	CCF	2.63	13.7	3.67	5.21	1.40	3.73	705	3.0
8/21/2002	CCF	2.04	5.82	3.95	2.85	1.94	1.47	1530	48.0
8/21/2002	CCF	1.88	15.9	1.95	8.46	1.04	8.15	545	10.0
5/15/2002	CCF	3.35	5.01	3.7	1.50	1.10	1.35	1033	49.0
8/21/2002	CCF	2.35	15.2	1.49	6.47	0.63	10.20	509	10.0
4/12/2001	LMB	5.28	7.74	---	1.47	---	---	431	30.0
5/19/2005	LMB	7.03	11.3	8.45	1.61	1.20	1.34	771	72.0
5/19/2005	SMB	5.42	6.54	6.93	1.21	1.28	0.94	680	89.0

Appendix 2. Selenium concentrations in whole body (WB), ovary and egg (Egg), and muscle plug (MP) samples taken from native and non-native fish species in the Colorado River.

Collection Date	Species[1]	WB Se ug/g DW	Egg Se ug/g DW	MP Se ug/g DW	Egg/WB	MP/WB	Egg/MP	WB Wt.(g)	Egg Wt.(g)
5/19/2005	SMB	4.19	5.96	3.67	1.42	0.88	1.62	381	36.0
5/14/2004	SMB	5.07	7.13	5.48	1.41	1.08	1.30	431	32.0
5/14/2004	SMB	4.9	8.81	7.7	1.80	1.57	1.14	469	47.0
5/10/2004	SMB	5.51	8	6.45	1.45	1.17	1.24	442	42.0
5/14/2004	SMB	7.82	11	11	1.41	1.41	1.00	181	7.0
6/3/2002	FHM	7.13	8.69	---	1.22	---	---	5	0.52
6/3/2002	FHM	7.64	8.8	---	1.15	---	---	4	0.49
6/3/2002	FHM	7.63	11	---	1.44	---	---	4	0.20
6/3/2002	FHM	7.18	8.57	---	1.19	---	---	4	0.48
6/3/2002	FHM	8.93	9.97	---	1.12	---	---	4	0.50

[1] Species: FHM=Flannelmouth sucker, BHS=Bluehead sucker, CCP=Common Carp, BT= Brown Trout, RTC=Roundtail chub, BG=Bluegill, BH=Black bullhead, CCF=Channel catfish, LMB=Largemouth bass, SMB=Smallmouth bass, FHM=Fathead minnow

Appendix 3. Selenium concentrations (ug/g DW) measured in muscle plugs taken from wild Colorado pikeminnow in the Colorado and Gunnison rivers during 2004 and 2005.

Fish ID	Date	River Mile	Length	MP Selenium	Egg[1] Selenium	Egg[2] Selenium	Egg[3] Selenium
PM4B1A	4/7/2004	156.5	747	8.05	8.6	16	
PM1F46	4/6/2004	162.7	712	7.04	7.8	14.1	
PM6C21	4/6/2004	164.3	671	5.53	6.6	11.2	
PM3002	4/6/2004	159	861	6.17	7.1	12.5	
PM3476	4/13/2004	153.3	585	5.87	6.9	11.9	
PM25D6	4/13/2004	154.7	658	5.94	6.9	12	
PM5A70	5/3/2004	167.3	834	4.97	6.1	10.2	
PM4B39	5/4/2004	168.7	690	7.08	7.9	14.2	
PM0B42	5/4/2004	167.7	595	4.99	6.1	10.2	
PM775A	5/10/2004	167.9	603	7.77	8.4	15.5	
PM0666	5/10/2004	GUN 1.2	715	7.45	8.1	14.9	
PM276E	5/10/2004	167.9	638	8.03	8.6	16	
PM4E43	5/14/2004	183	769	3.83	5.1	8	20.3
PM3405	5/17/2004	GUN 2.7	660	6.38	7.3	12.9	
PM7E34	5/17/2004	169.9	724	6.48	7.4	13.1	
PM4D72	5/18/2004	166.2	792	3.67	4.9	7.7	18.8
PM1C6F	5/18/2004	162.8	591	3.94	5.2	8.2	21.4
PM3A31	5/18/2004	159.4	782	5.77	6.9	11.7	
PM2D15	5/18/2004	165.2	850	4.56	5.8	9.4	
PM99C7	4/4/2005	159.1	745	5.54	6.6	11.3	
PM83F6	4/4/2005	159.1	604	7.7	8.3	15.3	
PM9B44	4/11/2005	169.8	474	6.24	7.2	12.6	
PM9070	4/4/2005	182.8	752	3.79	5.1	7.9	19.9
PM7BA6	4/7/2005	173.1	654	3.76	5	7.8	19.6
PM93AB	4/7/2005	169.2	579	6.21	7.2	12.5	
PM7559	4/4/2005	184.8	641	3.6	4.9	7.5	18.1
PM561F	4/19/2005	162.8	511	7.41	8.1	14.8	
PM1603	4/19/2005	158.5	817	6.76	7.6	13.6	
PMA580	4/19/2005	162.1	558	8.4	8.9	16.7	
PM6867	4/19/2005	162.1	660	7.4	8.1	14.8	
PM922D	4/19/2005	159	810	6.72	7.6	13.5	
PM12AE	4/21/2005	175.5	624	6.74	7.6	13.5	
PM9B27	4/21/2005	175.5	606	5.47	6.5	11.1	
PM6FEB	4/21/2005	173.1	683	7.08	7.9	14.2	
PM604F	4/22/2005	156.8	645	6.41	7.3	12.9	
PM8906	4/22/2005	156.8	620	5.84	6.9	11.8	

[1] Egg selenium concentrations estimated from muscle plug concentrations using Eq.3

[2] Egg selenium concentrations estimated from muscle plug concentrations using PS RTC Eq. (Table 2)

Appendix 3.cont'd Selenium concentrations (ug/g DW) measured in muscle plugs taken from wild Colorado pikeminnow in the Colorado and Gunnison rivers during 2004 and 2005.

Fish ID	Date	River Mile	Length	MP Selenium	Egg[1] Selenium	Egg[2] Selenium	Egg [3] Selenium
PM553A	5/16/2005	183.2	663	3.98	5.2	9.3	21.8
PM561F	4/19/2005	162.8	511	7.41	8.1	14.8	
PM1603	4/19/2005	158.5	817	6.76	7.6	13.6	
PMA580	4/19/2005	162.1	558	8.4	8.9	16.7	
PM6867	4/19/2005	162.1	660	7.4	8.1	14.8	
PM922D	4/19/2005	159	810	6.72	7.6	13.5	
PM12AE	4/21/2005	175.5	624	6.74	7.6	13.5	
PM9B27	4/21/2005	175.5	606	5.47	6.5	11.1	
PMC3B0	5/16/2005	183	638	4.41	5.6	9.1	

[1] Egg selenium concentrations estimated from muscle plug concentrations using Eq.3

[2] Egg selenium concentrations estimated from muscle plug concentrations using PS RTC Eq (Table 2)

[3] Egg selenium concentrations estimated from muscle plug concentrations using Buhl & Hamilton (2000)

Appendix 4. Selenium concentrations measured in razorback sucker muscle plugs taken from fish at large for at least eight months in the Colorado and the Gunnison rivers.

Fish ID	Date	River Mile	Length	Selenium	Egg Se[1]	Risk

			(mm)	(ug/g DW)	(ug/g DW)	Assess.
RZ0166	4/6/2004	165.7	430	5.3	8.3	low
RZ5368	4/6/2004	159.1	450	4.28	6.3	low
RZ6A5E	4/7/2004	156.7	455	4.84	7.3	low
RZ3574	May-04	GUN 3.0	413	9.49	17.6	mod
RZ235E	5/7/2004	153.4	435	4.52	6.7	low
RZ3D3D	5/13/2004	153.3	386	7.73	13.5	mod
RZ0243	5/13/2004	157.1	500	6.72	11.2	mod
RZ5005	5/13/2004	156.9	473	3.98	5.7	low
RZ0F6E	5/20/2004	153.7	456	8.55	15.4	mod
RZ1F0B	5/21/2004	174.4	411	4.91	7.5	low
RZ506E	5/21/2004	174.4	430	4.89	7.5	low
RZ7E73	5/25/2004	168.7	495	7.32	12.6	mod
RZ0133	7/12/2004	146.4	425	3.99	5.7	low
RZ1959	7/13/2004	131.8	421	5.89	9.5	low
RZ0166	7/19/2004	Redl. FL	443	4.62	6.9	low
RZ227F	7/21/2004	Redl. FL	457	5.99	9.7	low
RZ5368	4/5/2005	159.1	460	4.77	7.2	low
RZ3801	4/5/2005	159.1	322	5.8	9.3	low
RZ2159	4/6/2005	154.4	435	5.01	7.7	low
RZ7F5E	4/6/2005	154.1	380	6.22	10.2	mod
RZ247A	4/6/2005	154.1	354	3.73	5.1	min
RZ4054	4/6/2005	153.7	366	3.5	4.8	min
RZ3200	4/6/2005	167.7	476	6.33	10.4	low
RZOD68	4/7/2005	177	360	27.1	68.7	mod
RZ4B05	4/7/2005	175.8	396	6.11	10	low -mod
RZ2E63	4/7/2005	175.4	470	6.54	10.8	mod
RZ4DOA	4/7/2005	171.1	438	5.19	8	low
RZ3E04	4/11/2005	GUN 2.6	426	10.1	19.1	mod
RZ104F	4/11/2005	GUN 2.6	516	15.8	34.1	high
RZ7E72	4/11/2005	169.8	443	5.83	9.3	low
RZ5C2E	4/11/2005	167.7	433	8.53	15.3	mod
RZ4D6E	4/11/2005	168.8	464	12.1	24.1	high
RZ1C22	4/15/2005	162	406	10.1	20.3	high
RZ4039	4/20/2005	154.7	405	5.44	8.5	low
RZ0613	4/21/2005	176.3	444	4.53	6.8	low
RZ523B	5/16/2005	184.1	435	8.48	16	mod
RZ751B	5/17/2005	175.5	395	5.18	8.5	low
RZ0938	5/17/2005	176.5	349	4.17	6.1	low
RZ594D	5/17/2005	175.5	384	3.7	5.2	low
RZ166C	5/17/2005	172.2	455	18.1	40.9	high
RZ5D4D	5/17/2005	172	388	5.97	9.7	low
RZ1062	6/1/2005	154.2	443	15.2	32.5	high
RZ3D18	6/2/2005	152.7	435	6.89	11.6	mod

[1] Egg concentrations estimated from model developed from data in Hamilton 2001a&b

Appendix 4. cont'd   Selenium concentrations measured in razorback sucker muscle plugs taken from fish at large for at least eight months in the Colorado and the Gunnison rivers.

Fish ID	Date	River Mile	Length (mm)	Selenium (ug/g DW)	Egg Se[1] (ug/g DW)	Risk Assess.
RZF69C	6/2/2005	174.4	375	4.57	6.8	low
RZ084C	7/24/2005	Redl. FL	497	7.67	13.3	mod
RZ153F	8/2/2005	Redl. FL	395	9.42	17.5	mod
RZEBFA	8/16/2005	Redl. FL	445	6.94	11.7	mod
RZ194F	8/23/2005	Redl. FL	461	6.47	10.7	mod
RZ431A	8/29/2005	Redl. FL	395	9.96	18.7	low

1 Egg concentrations estimated from model developed from data in Hamilton 2001a&b